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Commentary

Future Directions and Research Priorities for Food Mutagens*

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This article is an expanded summary of the workshop discussions. Its objective is to add perspective and future orientation to the scientific symposium presented in the previous articles of this volume.

This symposium on formation of mutagens during cooking and heat processing of foods has covered the current status of the research effort and the significant findings to date. In order to provide an opportunity for discussion of additional research perceived as needed, future directions that should be addressed, and steps leading toward assessment of possible human health effects, a workshop attended by many of the symposium participants was held on the following day, December 19, 1984, at the Halekulani Hotel in Honolulu. This workshop was sponsored by the U.S.–Japan Environmental Panel on Mutagenesis and Carcinogenesis, and was co-chaired by Dr. F. J. de Serres, Associate Director of the National Institute of Environmental Health Sciences, and Dr. T. Sugimura, President of the National Cancer Center of Japan.

National Food Mutagen Research Programs

Dr. Sugimura discussed the Comprehensive 10-year Strategy for Cancer Control recently established by Prime Minister Nakasone. This program has components for research, education and cancer therapy and provides a framework for planning and implementing the Japanese cancer effort. Goals are set for future reduction of the cancer burden in Japan. This program could provide a mechanism for training and exchange of investigators between countries to develop cooperative research on food mutagen problems. A specific collaboration has just been initiated to evaluate the carcinogenicity of the cooking mutagen IQ in monkeys at a contract laboratory in the United States, with the synthetic chemical being supplied from Japan. The carcinogenicity studies performed to date in Japan on food mutagens have been single-dose experiments. However, it is important to carry out multilevel dose experiments, since the actual human intake of dietary mutagens/carcinogens is low level and chronic.

D. Longfellow of the Division of Cancer Etiology of the U.S. National Cancer Institute described the NCI effort on dietary factors in carcinogenesis as a significant new initiative. The first set of proposals addressing this area had just been received and were undergoing review. Therefore, the future shape of the program is not yet clear.

E. Zeiger of the National Institute of Environmental Health Sciences and National Toxicology Program described the history of the research on mutagens formed during cooking that is being carried out under an Interagency Agreement with the Department of Energy at Lawrence Livermore National Laboratory. The objectives of this project are: (a) to identify the mutagens produced in foods cooked under approximately normal household conditions, (b) to determine the mechanism of formation of these mutagens, (c) to assess the spectrum of genetic toxicity caused by the mutagens (with microbial tests and both *in vitro* and *in vivo* short-term mammalian bioassays), (d) to devise strategies to limit

*A preliminary Workshop Report was supplemented by many of the participants to create this article. The complete list of participants follows: (U.S.A.) F. de Serres, F. Hatch, J. Felton, M. Pariza, H. Mower, J. Weisburger, S. Thorgeirsson, E. Zeiger, L. Kolonel, D. Longfellow; (Japan) T. Sugimura, T. Matsushima, M. Nagao, S. Nishimura, S. Sato, M. Kuratsune, H. Kasai, H. Ohgaki, T. Hirohata; (Canada) W. Powrie; (Australia) R. Baker.

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or prevent mutagen formation, and (e) to estimate the risk to the population posed by mutagens in the diet.

Mutagens Formed during Cooking and Heat Processing

F. Hatch introduced the discussion of mutagens formed during cooking by presenting a list of topics that need further research or that have not yet been addressed to a significant extent.

(1) Are the same types of mutagens found in different cooked foods? Preliminary information from work at Livermore shows that when beef is cooked at a series of temperatures, a similar set of mutagens may be formed (based on chromatographic profiles), but that relative amounts of polar and less polar mutagens are variable. This study included barbecuing. Also, eggs fried at a rather high temperature contain IQ as a major mutagen, plus a mixture of several unidentified constituents (Bjeldanes, Grose et al., unpublished). Work on other meats and high-protein foods is not yet available.

(2) What is the nature and level of mutagens found during commercial processing such as canning? There is a remarkable paucity of information in this area. Only Krone, et al. and a few Japanese reports have dealt with processed foods. The preliminary information shows that certain fish species and canning procedures produce significant amounts of unidentified mutagens. Much more work in this area is called for and would make an ideal part of the subject for collaboration with the food industry.

(3) There may be food mutagens that have no or low mutagenicity in Salmonella, but which are active in other short-term genetic assays? There are no clear data at this time. An impression is that there is probably no short-term assay at the mammalian cell level with sufficient sensitivity to detect such mutagens in a screening mode. Also there is no definite guidance for extraction procedures for unknown types of mutagens in foods that offers hope of rapid enrichment to concentrations where such mutagens would be detectable.

(4) Total and specific mutagen intake from cooked foods should be measured in populations consuming different diets. Even for mutagens readily detectable with Salmonella, measurement of specific mutagen contributions will require major advances in methods of isolation and analysis to obviate the lengthy and laborious efforts now required. J. Weisburger added that attention should be given to the content of thermic and other mutagens in the diets of ethnic groups in the U.S. (e.g., Hispanic, Oriental) and religious groups such as Seventh Day Adventists and Mormons.

(5) Relevant model systems have been developed for study of the precursors and conditions required for mutagen formation. One such system, being studied by R. Taylor at Livermore, is based on soluble constituents of beef. Another model being studied in both Japan and Sweden, is based on heating mixtures of sugars, amino acids and creatine/creatinine. Mechanistic considerations for the latter have focused on nonenzymic browning (Maillard) reactions. However, the beef-based system and the chemical nature of the known thermic and pyrolytic food mutagens suggest that additional thermal mechanisms for formation of heterocyclic aromatic amines should be considered. The model system work also needs extension to development of measures to prevent or inhibit the reactions of mutagen formation. If feasible, mitigation measures should be operable without drastic changes in usual cooking methods.

(6) Pharmacodynamic studies of dietary mutagens are needed in rodents, primates, and humans. It is vitally important to understand the mode of action of these agents in different species. Detailed information is desired on reactions leading to activation of promutagens, potential detoxification reactions, cytochrome P450 types and inducers, and rates and routes of excretion. Exposure dosimetry via tissue binding or specific DNA adducts is important. However, more sensitive measurement techniques are needed, particularly nonradioactive (monoclonal antibodies–?) for human use.

(7) To the extent that it is feasible and ethical, human beings ingesting specific cooked foods or types of diets should be studied with short-term genetic assays and ultrasensitive biochemical analyses to provide data most relevant for risk assessment. An ethical intervention study might place subjects at a hypothetically lower risk under conditions where a higher risk is very improbable.

Mutagens in prepared foods and beverages were discussed by S. Nishimura and M. Nagao. A variety of instances of mutagen content have been observed, but identification is incomplete in most cases. For screening foods there is a need for improved procedures of isolation and analysis. The Blue Cotton adsorption-elution method shows promise for the rapid isolation of multiring aromatics because enrichment can be achieved faster than with other methods, including XAD resins.

Formation of Mutagenic Activity in the Digestive Tract and Excretion of Mutagens in Feces and Urine

H. Mower discussed the research needs with respect to mutagens found in urine and feces. Excretion of mutagens should be related to both the composition of the diet and the bacterial flora of the gut. Recovery of measured intake of dietary mutagens in the excreta should be determined; the chemical form, whether unchanged or in the form of metabolites, and the kinetics of excretion after ingestion are important pharmacodynamic data, particularly in humans. Interindividual variability in the processing of mutagens should be determined. Humans who are "mutagen-producers" should be identified and studied with respect to diet and intestinal flora. Protective factors in the diet might be identified by study of effects of specific foods or food classes on mutagen formation and excretion. The long-term implications of mutagen excretion should be evaluated

with respect to possible carcinogenesis in the bladder or colon.

F. de Serres proposed pharmacodynamic studies of the mutagens found in the GI tract. The absorption into the body, tissue distribution, and metabolic fate are not understood and may be of importance in assessing whether any possible hazard from these substances extends to internal tissues or is limited to the intestinal mucosa.

R. Baker presented the present state of knowledge and research needs in regard to urinary excretion of mutagens. Objectives of this research are: to determine whether dietary mutagens or factors formed in the digestive tract are excreted in urine; to determine what, if any, metabolic transformation has taken place prior to excretion, ie. are factors excreted unchanged or as conjugates or other metabolites; to determine the amount of substance excreted in urine, in relation to the ingested precursor. Use these data to derive further information about adsorption and/or metabolism; to use excretion data to determine what pharmacokinetic differences exist in human populations with respect to these mutagens. Are these pharmacokinetic differences related to cancer risk for specific sites?

Significant research findings have been as follows. Dolara et al. (Mutat. Res. 79: 213–221, 1980) studied the excretion of beef-extract mutagens in rats after systemic administration PO or IP. They reported 2.5% of ingested mutagens were recovered in urine, based on biological assays with Salmonella tester strains.

J. Weisburger has recently reported that up to one-third of IQ administered to rats was excreted unchanged in their urine, based on HPLC analysis of radioisotopically labelled IQ. It was possible that a small proportion of this material was excreted as a glucuronide conjugate.

Using biological assay techniques, it was previously estimated that up to one-third of ingested mutagenic activity was excreted unchanged in human urine after fried bacon or pork meals (Baker et al., Cancer Letters 16: 81–89, 1982). Since then Sousa, Nath, and Ong (Environ. Mutagen. 6: 476, 1984) have confirmed that there is also considerable excretion of urinary mutagenic activity after beef meals. However, quite different results have been reported by Dolara et al. (Cancer Letters 22: 275–280, 1984) who found that only about 0.6% of the ingested dose was recovered in human urine.

Baker et al. have now repeated and extended the original observations. This study resulted in a re-estimation of 24 hr excretion levels at between 2 and 10% of ingested activity. This "downward" revision of earlier estimates was largely due to a more efficient extraction procedure for measuring the mutagenic content of ingested bacon. The new estimates of urinary excretion are extremely reliable, since "spiked" urine was tested and demonstrated the XAD-2 recovery procedure to yield approximately 98% of bacon mutagen.

The current data base on urinary excretion of mutagens is inadequate in that, apart from meat mutagens, we have little or no information on other food mutagens, i.e., excretion of clastogenic activity after fruit ingestion. We have no information on excretion of beverage mutagens, although we do know that mutagenic activity in wine is not excreted unchanged in urine (Baker, unpublished). Thus, wine and bacon mutagens probably behave quite differently in the body.

Although we know that urinary excretion of meat mutagens is very variable (Baker et al., these proceedings) we do not know what factors account for this inherent variability. This problem is exacerbated by the fact that all of the human studies involve biological assay procedures. We have very few analytical data on food mutagens in body fluids, except for one or two animal studies.

The principal future needs are: chemical analysis of dietary mutagen excretion, e.g., by HPLC, both in animal and human studies; studies of possible excretion of conjugated forms of mutagens, particularly by some individuals in the population; investigation of a range of foods and beverages, both as contributors to a dietary mutagen load, and as protective factors (i.e., preventing absorption of ingested mutagens).

Carcinogenesis Bioassays

S. Sato discussed assays for carcinogenic activity. Recent experiments with multiple-agent exposures to the heterocyclic food mutagens have already shown that the combined effect is more than additive. He discussed the need for more than single-dose experiments when a single agent is tested. There is also a need for detailed dose-response curves for representative food mutagens, and a need to know the shapes and types of dose-response curves in order to do risk estimation. He discussed the need for short-term *in vivo* assays to assist in selection of chemicals that are active *in vitro* for the traditional rodent bioassay for cancer.

J. Weisburger pointed out that human consumption of dietary mutagens is (presumably) accompanied by other food constituents that may exert promoting effects. Dose-response carcinogenesis bioassays should be conducted under conditions where promotion can be assessed as a variable.

S. Thorgeirsson commented that the heterocyclic food mutagens cause multiple primary tumors when fed to rodents. The tumor spectrum includes organ sites, such as breast and colon, that are major sites for human tumors. Since the etiology of most human cancers is unknown, it is reasonable to propose a major research effort on the possible etiological role of these food-derived mutagens and carcinogens in human cancer.

Modulation

Modulation of mutagenic/carcinogenic activity was discussed by M. Pariza. Where we identify modulators in *in vitro* assays, we need to have a more thorough analysis of mechanism. He also proposed that we do long-term bioassays to determine whether *in vitro* modulators are active *in vivo*, and research to determine

whether modulators of mutagenic activity are also modulators of carcinogenic activity. Some agents can modulate mutagenesis in either direction depending on the chemicals that are used to induce the S-9 fraction, as well as the levels of exposure to the modulator agent. A study currently in progress at the University of Wisconsin is showing that the fat content in the diet may be less important than the net energy intake in influencing the frequency of induced mammary tumors.

F. de Serres suggests further study of dietary constituents that are not in themselves mutagenic, but which can modulate the activity of known dietary mutagens such as the mutagens derived from cooking, the flavonoids, or the mycotoxins. Are there dietary constituents or other safe additives that will modify metabolism by enhancing detoxification reactions in animals or man?

Epidemiology

In the discussion on epidemiology, L. Kolonel summarized current work by noting that very little epidemiologic work has been done which bears directly on exposure to mutagens and cancer risk. Studies on fecal mutagen levels in Japanese populations at different risks for colon cancer by H. Mower et al. (Cancer Res. 42: 1164–1169, 1982) represent one attempt to address this issue. Studies which associate the consumption of broiled fish with stomach cancer risk in Japan, as presented at this Symposium by Kuratsune and by Hirohata, suggest the possibility of a role for mutagens in these foods. However, the similar findings for dried/salted fish and pickled vegetables indicate that components of the diet other than mutagenic byproducts of cooking may be responsible.

There was considerable discussion of the types of epidemiologic studies that are needed to elucidate further the role of dietary mutagens in the etiology of human cancer. Kolonel emphasized that most epidemiologic studies have looked at foods or their nutrient content without regard to method of preparation, other than to separate raw from cooked foods in some instances. Studies which clearly distinguish among different cooking methods for the same foods are needed. Such studies should build on the results of laboratory research which has identified specific foods and cooking practices as sources of dietary mutagens. Laboratory research has also associated mutagens with tumors outside the gastrointestinal tract in rats; thus, epidemiologists should not limit their studies of mutagens to gastrointestinal cancers. The importance of studying populations with particular dietary practices, such as the Seventh Day Adventists, was stressed by Kuratsune in his remarks. J. Weisburger added that vegetarian groups do fry some of their foods and that few data are available on the mutagen content of such foods.

Close collaboration between epidemiologists and laboratory workesr is necessary if future research is to be optimally productive. From the epidemiologist's view, dietary mutagens can be sought from two sources: foods, as prepared, prior to ingestion; or body tissues and waste products (notably urine and feces). In the former instance, epidemiologic studies can be designed to identify specifically prepared foods associated with increased cancer risk that can be examined in the laboratory for mutagenic content. Epidemiologic studies can also be designed to test hypotheses based on the findings of laboratory mutagenicity assays. The interchange of information between the two approaches can be informative. For example, if a mutagen in a certain food is clearly identified in laboratory assays as a potential carcinogen but epidemiologic studies indicate that the consumption of this food (accounting for preparation methods) does not distinguish between persons at high and low risk for cancer, then the mutagen is unlikely to be important in the etiology of human cancer. Target organ specificity must be considered in this regard, however. On the other hand, if the laboratory scientists identify a common mutagen of potential significance in a variety of foods (cooked in a particular way, perhaps), then this might suggest a grouping of foods for analysis in epidemiologic studies that would not otherwise have occurred to the researchers; a positive finding might thus be found that otherwise might have been missed.

In the case of laboratory assays of body tissues or excreta, there are limitations to the epidemiologic approaches that can be used. Aggregate correlational analyses based on representative sampling in populations at different levels of risk for one or more cancers can be useful (Mower et al., Cancer Res. 42: 1164–1169, 1982). Similarly, prospective cohort studies can be carried out in which specimens are collected at the outset from a very large number of healthy people. To reduce the costs of analysis, the nested case-control approach can be used in which only the specimens from the incidence cases which subsequently occur and from suitable matched controls are analyzed. Regular case-control studies, on the other hand, may be of limited usefulness if there is any reason to suspect that the disease process would either have altered eating habits or metabolic processes involved in the endogenous production of mutagens from dietary components (e.g., colon cancer cases might have altered gastorintestinal flora either from the disease itself or a change in dietary habits; and the altered flora may result in a different distribution of mutagenic bile acid metabolites in the intestinal tract). A final epidemiologic approach would be intervention studies in which subjects would be randomized to either adopt a mutagen-reducing diet (e.g., elimination of broiling and frying as methods of food preparation) or not. In order to reduce the followup period necessary to observe a statistically significant effect, such a study might be based on precursor lesions in a high risk group (e.g., post-polypectomy patients who would be followed for the recurrence of colonic polyps). The latter approach would be best justified, however, if the mutagenic substances were suspected of playing a role in one of the later stages of carcinogenesis.

With respect to the intiation phase of carcinogenesis, J. Weisburger pointed out that repeated exposure throughout life may not be necessary. Genotoxic carcinogens, apparently exemplified by the thermic food mutagens, might only require limited exposure during the early growth phase of an animal or human in order to initiate the process. Subsequent promotion by dietary or other environmental factors may complete the carcinogenesis. Thus, epidemiologic studies limiting their attention to the habitual diet of adult life could fail to detect relevant effects.

Societal Implications of Food Mutagens

After the foregoing topical reviews of the state of knowledge of dietary mutagens, with emphasis on those arising from cooking and heat processing, the broader societal issue posed by these mutagens was addressed in the workshop. The question arises of when the laboratory scientists and epidemiologists working in this field will be ready to present their findings to the food processing and service industries, to government agencies, and to the general public in a coordinated fashion. Examples were cited where mutagenicity data for marketed products were used to reduce or eliminate mutagenic activity: specifically, Xerox toners and hair dyes. The importance of truthfulness and rational management of information by scientists was stressed.

The workshop attendees were not able to develop a generally acceptable plan for presenting the status of this subject. They felt that continued publication of new material in peer-reviewed journals is the most suitable mechanism at this time. There was general agreement that a more extensive database on short-term genetic assays beyond those in Salmonella, especially from *in vivo* animal tests, is needed as a foundation for evaluation of potential human health hazards. S. Thorgeirsson urged that this database be centralized, readily accessible to the scientific community, and maintained current.

S. Thorgeirsson also called attention to a recent valuable symbiosis between industry and universities with respect to several kinds of research, notably in the field of recombinant DNA. He recommended a similar approach joining the food industry, government agencies and laboratories, and universities in a major research effort on the role of food-derived mutagens and carcinogens in human health, and on possible preventive measures for eliminating these harmful agents or mitigating their effects.

There was agreement that the workshop had provided a fine opportunity for discussion of broader issues than are appropriate in conferences and symposia. The format of this workshop was experimental, owing to the diverse background of the participants and some uncertainty on how to address the subject matter. It was, in fact, a stimulating session, and there was a consensus that periodic workshops of this type would be valuable for maintaining a continuing review and perspective of the status of research on food mutagens.